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THE UNIVERSITY OF ALBERTA

THE EFFECT OF CERTAIN HORMONES ON THE TISSUE RESPIRATION AND PHOSPHORUS METABOLISM OF FEMALE RAT SEX ORGANS

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS OF
THE DEGREE OF MASTER OF SCIENCE

FACULTY OF PHARMACY

by

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EDMONTON, ALBERTA, CANADA

September, 1964

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled " The Effect of Certain Hormones on the Tissue Respiration and Phosphorus Metabolism of Female Rat Sex Organs", submitted by MAN SEN YONG in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

An investigation was undertaken to determine the effect of estrus and certain hormones on the tissue respiration and the phosphorus metabolism of sex organs in the female rat. Tissue respiration was determined by the Huston-Martin technique (29) and the phosphorus metabolism was measured by the incorporation of radioactive phosphorus into the acid soluble phosphate fraction of the tissue.

No significant difference was observed between the ovarian and the uterine oxygen consumption in normal female rats during estrus and anestrus.

Norethindrone increased the oxygen consumption and the radioactive phosphorus incorporation of the ovary, but it had no effect on the oxygen uptake of the uterus and appeared to decrease the radioactive phosphorus incorporation in the uterine tissue in normal female rats.

Hypophysectomy caused insensitivity of the ovarian tissue to norethindrone as evidenced by the lack of enhancement of oxygen consumption and radioactive phosphorus incorporation. It is suggested that norethindrone exerts its effect on the ovary indirectly through the hypothalamo-hypophyseal complex. In the uterus both the oxygen consumption and the radioactive phosphorus uptake were markedly increased in hypophysectomized rats by norethindrone treatment.

In normal female rats, gonadotropin had no appreciable effect on the oxygen consumption of the ovary and the uterus, but it did increase the radioactive phosphorus uptake in these tissues. Both the oxygen consumption and the radioactive phos-

phorus incorporation in the uterus were significantly enhanced in hypophysectomized rats by gonadotropin. No changes in the ovarian radioactive phosphorus uptake was observed and the ovarian oxygen consumption was significantly depressed in gonadotropin treated hypophysectomized rats.

The oxygen consumption and the radioactive phosphorus uptake in the uterus were found to be least after ovariectomy. Estradiol dipropionate dramatically restored these metabolic deficiencies.

The possible mechanism of the effects of these hormones on the metabolism of sex organs is discussed.

ACKNOWLEDGEMENTS

The author gratefully acknowledges the valuable assistance, encouragement and criticism given by Dean M.J. Huston and Dr. B.E. Riedel throughout the course of this study.

He wishes to thank the Warner-Chilcott Company for offering the Warner-Lambert Research Fellowship to him.

He is grateful to the Parke Davis and Company for supplying the Norethindrone and Gonadotropin used in these experiments.

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I. INTRODUCTION:

The importance of the ovaries in maintenance of normal sexual function has long been recognized. The hormonal control of the female reproductive system by ovaries (1,2) and anterior pituitary (3-6) was established in the early years of this century. Subsequent studies lead to the isolation of estrogenic hormone by Doisy *et al.* (7,8) and Butenandt (9). The hormonal function of corpus luteum was first discovered by Fraenkel (10) and later confirmed by Corner and Allen (11) and the active substance progesterone was isolated in 1934 (12). These, together with the discovery and isolation of several gonadotropic hormones (13-20), opened a new era in endocrinological research. Extensive studies have been made by means of various approaches in attempts to elucidate the interrelationship between these hormones and the basic mechanism underlying their actions on the target organs. The measurement of oxygen consumption together with phosphorus uptake has been considered to be one of the best ways to study the effects of these hormones on the target organs either *in vitro* or *in vivo*.

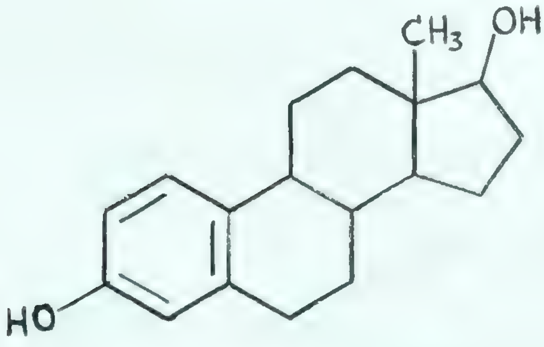
During the past few years a number of synthetic progestational steroids have been introduced into control of conception and found to be highly effective (21-28). Although extensive studies have been undertaken on their contraceptive properties, less attention has been paid to their effects upon the metabolism of target organs.

The purpose of the present study was to determine the effects of norethindrone (norlutin) on the metabolism of ovaries and uteri of rats under various endocrine states as measured by tissue respiration and radioactive phosphorus uptake using methods developed by previous workers in this laboratory (29-31). In addition, the effects of estradiol on the uterus of the ovariectomized rat and the effect of gonadotropin on the ovaries and uteri of normal and hypophysectomized rats were also noted.

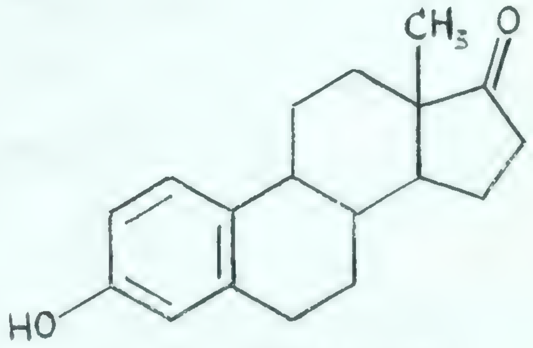
II. LITERATURE SURVEY:

A. Ovarian Hormones and Gonadotropins.

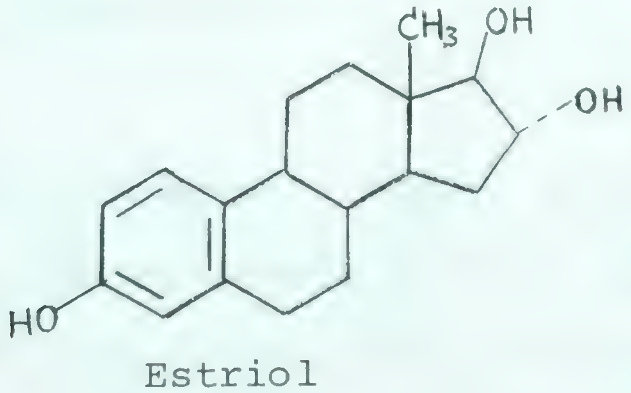
Estrogens and progesterone are the main hormones found in the ovaries in various species. They are steroids and may be classified chemically as derivatives of cyclopentanoperhydrophenanthrene. There are four principal naturally occurring estrogens, namely estradiol-17beta, estrone, estriol and 16-epi-estriol. Estrone was isolated by Doisy *et al*. (7,8) and by Butenandt (9). The most potent substance, estradiol-17beta, was first obtained by reduction of estrone (32), and later isolated from ovaries (33). Estriol, a metabolite of either estrone or estradiol-17beta was obtained from urine (34), whereas 16-epiestriol is derived mainly from placenta (35). A minute amount of estrogens can also be demonstrated in testis (36) and adrenal cortex (37). The structural formulae of the three naturally occurring ovarian estrogens are shown:



Estradiol



Estrone

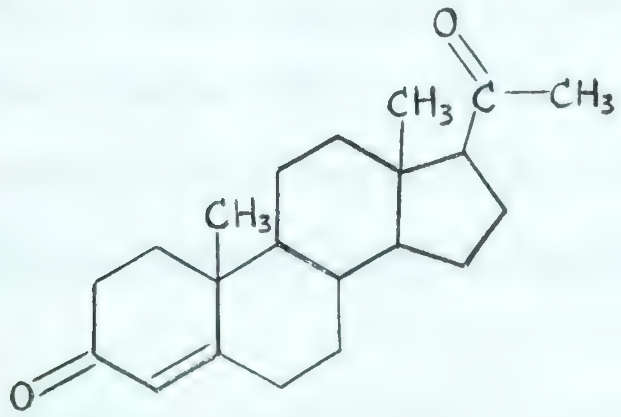


Estriol

The estrogens are unique among the steroids in that the A ring of the nucleus is aromatic and also phenolic due to the presence of an hydroxy group at C_3.

A number of synthetic nonsteroidal estrogens have been prepared. These include diethylstilbestrol, dienestrol (38), hexestrol, benezestrol (39), tri-p-anisylchloroethylene (40) and phenylethylene (41).

Fraenkel first demonstrated the importance of the corpus luteum in maintenance of pregnancy in rabbits (10). Corner and Allen further confirmed this observation (11) and pointed out that corpus luteum was necessary for the preparation of the uterus for a fertilized ovum (43). Progesterone, the hormone of corpus luteum, was consequently isolated in 1943 (2). Like estrogens, progesterone has been shown to be present in adrenal glands (44) and placenta (45). It is a diketone. The structure is as follows.



Progesterone

Gonadotropins represent a number of hormones elaborated by the anterior pituitary which have an important function in initiating puberty and maintaining and regulating the activity of the sex glands. A gonadotropin is also produced by the placenta. The effects of pituitary implants or extracts on the

function of gonads have been demonstrated in the early years of this century (3-6). Hisaw and Leonard separated pituitary extracts into two components which exerted different kinds of hormonal activity (13). These hormones have been designated as follicle stimulating hormones (FSH) and interstitial cell stimulating (ICSH) or luteinizing hormone (LH). Both FSH and LH have been obtained in pure form from the pituitary of sheep and hog (14-18). They are glycoprotein, the carbohydrate moiety being hexose and hexosamine. Another gonadotropin of pituitary origin is lactogenic hormone, better known as prolactin. Although it is primarily related to lactation, its effects upon the corpus luteum (46) and its effect on the production of progestational hormone from luteal tissue (47) have been firmly established. Prolactin has been isolated in pure form (19,48). It differs from other gonadotropins in containing no carbohydrate moiety. The discovery of gonadotropic factor in the urine and serum of pregnant women lead to the isolation of another gonadotropin (HCG) from placental tissue (20). It is a polypeptide. There is another gonadotropin obtained from the serum of the pregnant mare, known as PMS which shows similar activity.

B. The Influence of Ovarian Hormones on Tissue Respiration.

Extensive studies have been made upon the effect of estrogen on the metabolism of the uterus of various species. David (48) studied the oxygen consumption of mouse uterus at various stages in the estrus cycle and found no significant difference between diestrus and estrus, and he was unable to show a definite rise in oxygen consumption in natural estrus or when

estrus was induced in the castrated animals. However, Khayyal and Scott (49) found that the oxygen consumption per unit weight of uteri of mice and rats showed a cyclical variation and remained uniform during the greater part of the cycle. Kerly (50) has reported that anaerobic glycolysis and oxygen consumption of isolated rat uterus are highest during pro-estrus and lowest at the end of estrus and the changes in respiration are not so pronounced as those in anaerobic glycolysis. In contrast, the aerobic glycolysis was lowest during pro-estrus and highest immediately after estrus. However, her results were not conclusive since pro-estrus values were found in some rats at the beginning of estrus. MacLeod and Reynolds (51) demonstrated that estradiol increased the rate of oxygen uptake of uteri from ovariectomized rabbits. Pincus and Graubard (52) found that six hours after intraperitoneal injection of estrogenic hormone to immature rats, no large change in respiration was observed. Carroll (53) studied the changes in the oxygen consumption of uterine tissue from ovariectomized rats with time after injection of 50 μg. of estradiol dipropionate. A slight increase at six to twelve hours was found. Marked increases of QO_2 were not observed until 24 hours after injection and a peak was reached by 45 hours. Kerly (54) was able to correlate the rate of anaerobic glycolysis and the growth of uteri from ovariectomized rats after injection of estrone. She also found a great variation in the rate of anaerobic glycolysis and oxygen consumption of uteri from castrated rats.

C. Hypothalamus Pituitary-Ovarian Interrelationship.

After it had been shown that ovulation could be induced

in the estrus rabbit by applying an electric current to the head (55) or directly to the hypothalamic tuberal and preoptic regions (56,57) considerable information was accumulated which reveals that the release of pituitary gonadotropin is stimulated via the central nervous system. By using the remote-control method for stimulation, Harris (58) studied the effect of electrical stimulation in different areas of the hypothalamus and hypophysis of rabbits and suggested that some of the nervous reflex-path normally excited by coitus traversed the hypothalamus to stimulate the release of luteinizing hormone from the adenohypophysis. Saul and Sawyer (59) have recently delineated an elliptical area extending from the rostral end of the mammillary body to the median eminence, stimulation of which consistently evokes ovulation. The method by which the stimulus is transmitted from the hypothalamus to the anterior pituitary gland has received much attention, and the following facts seem pertinent. First, the pituitary stalk seems to be the anatomical structure involved, since section of the stalk with steps taken to prevent regeneration, or pituitary transplantation to a distant site, results in ovarian atrophy. Secondly, the pituitary stalk contains two prominent structures linking the hypothalamus and the gland. The hypothalamo - hypophyseal nerve tract passes from the hypothalamus to the gland, to end, very largely in the neural lobe. Once cut, this tract does not regenerate. The hypophyseal portal vessels carry blood from capillaries in the median eminence of the tuber cinereum to the sinusoids of the pars distalis. It has been established that a blood supply derived from these vessels exerts a profound effect on anterior pituitary activity. The hypothesis has been put forward that the hypothalamus maintains and regulates

anterior pituitary activity by means of a neurohumoral mechanism acting through this vascular system (60). Recently it has been shown that hypothalamic extract made from mature rats or castrated rats induced an increase of gonadotropin content in cultivated anterior pituitary cells (61). In addition, there has been a speculation that the hypothalamic area which activates release of ovulating hormone is the same as that which chronically holds in check the release of prolactin (62). Kanematsu and Sawyer (63) studied the effects of intrahypothalamic and intrahypophyseal estrogen implants on the pituitary gonadotrophic cells in ovariectomized rabbits. Their results suggested that the inhibitory action of this hormone on gonadotropic cells is exerted indirectly via the hypothalamus. However, Bogdanove (64) was unable to obtain conclusive evidence in ovariectomized rats. Arai (65) demonstrated that in estrogen induced persistent-diestrus rats, the content of LH of the anterior pituitary and of LH-releasing factor of the hypothalamus are significantly lower as compared with those in normal female rats and suggested that prolonged treatment with this hormone seems to disturb irreversibly the regulatory mechanism of the production and release of LH-releasing factors by the hypothalamus.

D. The Effects of Ovarian Hormones on Phosphorus Metabolism in Target Organs.

Grauer *et al*. (66) studied the influence of estradiol on radioactive phosphorus uptake by the uterus and observed that there is increased binding of the administered P^{32} within the uteri of animals treated with estradiol. Both acid soluble and acid insoluble phosphate fractions in the hormone treated

rats showed low activity in the first few hours followed by a rapid increase after 24 to 48 hours which was well maintained through 72 hours. Walaas and Walaas (67) reported that in uterine muscle the specific activity of inorganic phosphate in ovariectomized and estrogenic treated animals was of the same order of magnitude without any significant difference. However, an increase in the specific activity of intracellular acid soluble organic phosphorus compounds was found in estradiol treated ovariectomized rats. In the acid insoluble fraction of uterine muscle low specific activity was found in both groups. They also observed a significant increase in glucose utilization in isolated uterine muscle of ovariectomized rats after estradiol benzoate administration (68), and they suggested that the increased turnover of glucose in the hexokinase reaction would explain the increased incorporation of P^{32} into the organic acid soluble fraction of uterine muscle after hormone treatment. Borell (69) came to a similar conclusion that estradiol benzoate markedly increases the metabolism of easily hydrolysable phosphate esters while progesterone accelerates the turnover of the slowly hydrolysable phosphate in rabbit's endometrium.

In addition, estrogen has been shown to increase the incorporation of several amino acids such as glycine (70), serine (71) as well as formate (72) into the protein of surviving uterine segments. That histamine might actually serve as the mediator of estrogen action has been speculated (73,74) and recently Szego and Lawson (75) were able to show an augmentation of incorporation of radioactivity derived from isotopic amino acids into uterine protein, lipid and the nucleic acids following intraluminal administration of histamine. It has been reported that both estradiol

and progesterone increase the incorporation of P^{32} into the phospholipids and nucleic acids of myometrium (76). Gorski and Mueller (77) observed that estradiol caused a dramatic expansion of certain uterine uridylic nucleotide pools during the first four hours of treatment, but had relatively little effect on adenosine triphosphate. Furthermore, it has been indicated by Wilson (78) that the enhancement of synthesis of certain RNA components and protein in uterus by estrogen resulted from increasing chromosomal RNA synthesis. Gorski and Nicolette (79) also noted that one of the earliest responses to estrogenic hormone treatment is an increase in RNA synthesis. More recently, Hamilton (80) studied the sequences of RNA and protein synthesis during early estrogen action using specific inhibitors such as actinomycin D and puromycin. He suggested that estrogen induces or activates protein synthesis in uterus through some unknown mechanism which underwrites DNA-dependent RNA synthesis.

E. The Effects of Gonadotropins on the Metabolism of Female Sex Organs.

Gonadotropins exert a stimulatory effect on the female reproductive organs. Thus, injection of various gonadotropins or pituitary implantation into hypophysectomized or immature animals markedly enhances follicle growth (81-84), the proliferation and repair of thecal and interstital tissue (83,85),and stimulatesovulation and the formation of corpora lutea (83,86). During the past decade, considerable work has been done in attempting to explain the basic mechanism underlying their actions either *in vivo* or *in vitro*. The effects of luteinizing

hormone (LH) on the ascorbic acid content of the ovaries has received much attention (87,88), and this has been used to assess the activity of luteinizing hormone (LH) (89,90). Biddulph _et_ _al_. (91) studied the activation of succinic dehydrogenase and adenosinetriphosphatase in corpora lutea, ovarian interstitial tissue and endometrium in gonadotropin treated rabbits. Armstrong (92) has reported that purified LH exerts a pronounced stimulatory action upon the rate of _in_ _vitro_ glucose uptake by slices of luteinizing rat ovaries, but has no effect when added directly to the incubation medium. FSH and prolactin produced no such effect. In addition, LH increases the production of lactic acid from added glucose under both anaerobic and aerobic conditions (93). Ahrén _et_ _al_ (94) studied various gonadotropins on the metabolism of isolated rat ovaries and found that FSH consistently and significantly stimulates the accumulation of C^{14} labelled alpha-aminoisobutyric acid (AIB) into the isolated ovaries. Neither ovarian glucose uptake nor oxygen consumption was stimulated by FSH. In contrast, LH was found to be ineffective in stimulating AIB-C^{14} accumulation but increases the glucose uptake in the ovaries. Chorionic gonadotropin administration to normal rats enhanced protein synthesis and P^{32} uptake by the ovary, but decreased cholesterol, glycogen and ascorbic acid. Such biochemical alteration suggests that gonadotropic hormones stimulate the ovary to increase tissue growth and protein synthesis and that carbohydrate was supplying at least some of the energy necessary for the synthesis (95). Callard and Leathem (95) found that 8-azaguanine decreases the chorionic gonadotropin induced lactic acid accumulation in mouse ovaries but has no effect on lactic acid production in uterus or

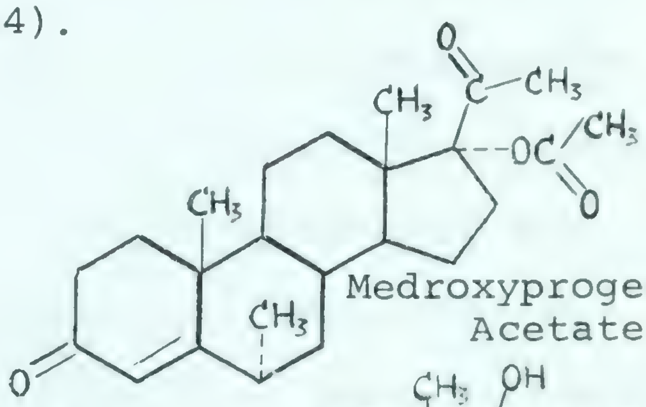
in unstimulated mouse ovaries. Gonadotropins with follicle stimulating activity have been shown to enhance the glutamic oxalacetic transaminase activity in the ovaries and uterus (96) and this effect is dependent on the reactivity of the ovaries to the exogenous gonadotropins (97).

F. Progestational Oral Contraceptives.

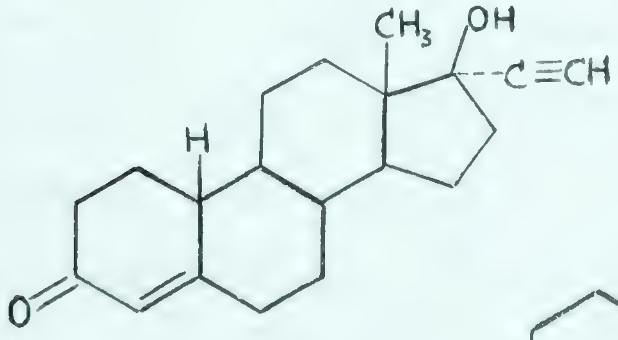
1. Chemistry.

The introduction of a series of orally effective progestogenic agents in the past few years (21,22),(98-100) not only initiated a new era in conception control, but also gave rise to a new field of studies toward the actual mechanism in the procreative process.

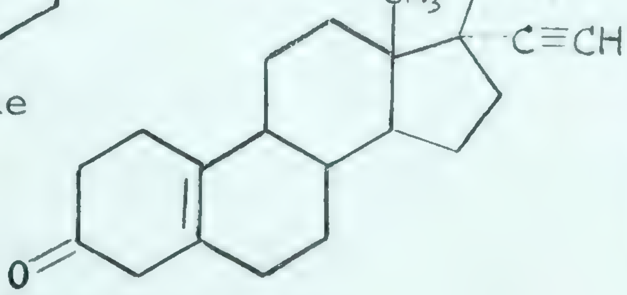
All of such progestogenic agents are synthetic steroids and with few exceptions can be classified into two groups: derivatives of progesterone and derivatives of 19-nortestosterone. Despite the fact that numerous compounds have been synthesized and screened for activity in experimental animals, only a few compounds, namely norethindrone, norethynodrel, medroxyprogesterone acetate and ethynyl estrenol were recommended for actual practice in human birth control (24-26),(101-104).



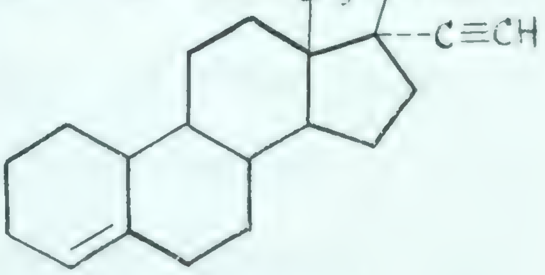
Medroxyprogesterone Acetate



Norethindrone



Norethynodrel



Ethynylestrenol

2. Physiological Actions.

These synthetic steroids, no less than normal hormones, possess a wide range of biological activities. Although these progestogens were originally introduced as agents for aiding fertility by their action on the endometrium (21,98), they are now being successfully used to limit fertility by inhibiting ovulation. Besides these actions, the progestogens have a number of actions which are exhibited in varying degree by the different compounds. They have anabolic and fluid retaining properties, some androgenic, myotrophic activity (105), mild corticoid and estrogenic activity (105,106), gonadotropin inhibitory (107,108) and antiestrogenic effects (109), an influence on water and electrolytes excretion (101,110) and on mating behavior (111).

3. Mechanism in Suppression of Ovulation.

It has been well known that both parenterally and orally effective progestational agents suppress ovulation in both experimental animals and humans. The degree of suppression, of course, varies from compound to compound and is governed by dose level. The mechanism involved is still undetermined , however, it is generally believed that the inhibition is brought about by either decreased secretion or reduced rate of synthesis of gonadotropin in the pituitary (107,108),(112-114). Both norethindrone and norethynodrel have been shown to reduce urinary pregnandiol excretion (23). Brown and Blain (115) suggested that it is possible that the inhibitory action of progestational agents on the pituitary is due to their conversion to estrogen in the body rather than to the effect of the com-

pounds _per se_. Loraine (116) studied the effect of progestational steroids on the urinary excretion of gonadotropin in human subjects. The findings suggest that progestational compounds are less active inhibitors of pituitary function in human subjects than are estrogens. Taylor and Klibanoff (117) studied the effects of norethindrone acetate in women. They found that ovulation may still occur below a critical dose level. However, due to the effect upon endometrium, the drug may still prevent conception. Holmes and Mandl (118) reported that in rats, prolonged treatment with norethynodrel at a dose level sufficient to cause sterility did not consistently block ovulation. They also noted that the pituitary gland of the treated animals was heavier than that of their controls due largely to an increase in the volume of tissue occupied by chromophobe cells. Munshi (119) found that norethynodrel suppresses the estrus cycle, causes a decrease in ovarian weight and cessation of corpora lutea formation in rats. Eckstein and Mandl (120) reported that the sensitivity of ovaries of immature female rats to exogenous gonadotropins was somewhat enhanced by high dosage of norethynodrel. Brown _et al_. (121) indicated that certain of the nor-steroidal progestogens produce their antifertility effect by acting directly on the ovary without the intervention of the pituitary gland. Recently, Overbeek and de Visser (122) found that ethynyl estrenol (lynestrenol) prevents ovulation by inhibiting FSH release from the pituitary gland so that there are no ripening follicles ready to respond to LH, while 6-alphamethyllynestrenol inhibits ovulation by abolishing LH release, thus preventing the final maturation and rupture of normally

developing follicles. Lynestrenol also was found to decrease markedly the urinary estrogen content (123). Medroxyprogesterone acetate has been shown to delay implantation in rats. This suggests that the drug may suppress the synthesis or the release of luteinizing hormone from the pituitary gland and thus prevent the production of sufficient endogenous estrogen to cause implantation (124).

Lunenfeld et al. (113) found that a mixture of medroxyprogesterone acetate and a minute amount of ethinyl estradiol administered in conjunction with gonadotropins inhibited the ovarian response to these exogenous gonadotropins with a resultant depression of excretion of urinary estrogens and urinary pregnandiol. Edgren and Carter (125) suggested that ovulation inhibitory action of synthetic progestational agents appears to be through effects on the hypothalamic centers controlling pituitary function. Lakshman and Nelson (126) studied the chronic effect of norethynodrel on various organs of the rats. A marked decrease in ovarian weight was observed. But surprisingly there is little effect upon the pituitary gland. This led them to speculate the norethynodrel might exert its pituitary effect on the neurohumoral system, which in turn affects the hypothalamo-hypophyseal complex to decelerate the secretion of gonadotropins.

III. METHODS AND MATERIALS:

A. Animals.

Female Sprague-Dawley virgin rats weighing from 150 to 275 Gm. were used in all experiments. They were fed with dog food pellets and tap water *ad libitum*.

In certain experiments, rats spayed two weeks before treatment, and hypophysectomized rats were used.

Bilateral ovariectomy was performed by the dorsal approach under light ether anesthesia. They were maintained on the same diet as those of normal animals.

Rats hypophysectomized by the parapharyngeal route were obtained from Hormone Assay Laboratory Inc., Chicago. They were received two days after surgery and were fed bread, oranges and carrots, plus drinking water containing one percent sodium chloride *ad libitum*. They were used in experiments within a one week interval after arrival.

B. Experimental Design.

The experimental work was designed as follows:

Part I: The study of the relationship between estrus and anestrus to the oxygen consumption of ovaries and uteri in normal rats. Estrus and anestrus was determined by the classical vaginal smear method. The appearance of full cornified vaginal epithelium in the animal was considered as evidence of the estrus stage, whereas a mainly leucocyte dominated vaginal epithelium was taken as evidence of the anestrus stage. Vaginal smears were taken ten minutes before sacrifice of the animal.

Part II: The study of the effect of norethindrone on the oxygen consumption, phosphorus concentration and radiophosphorus

incorporation of ovaries and uteri of the intact and hypophysectomized animals. Norethindrone (Norlutin, Parke Davis and Co.) was administered orally to the intact or hypophysectomized rats in 5 mg. per day dosage for two days. The control animal received no treatment.

Part III: The study of the effect of gonadotropin on intact and hypophysectomized animals. A preparation which combines chorionic and pituitary gonadotropin activity (Synapoidin, Parke Davis and Co.) was injected intramuscularly in dosage of 7.5 SU daily (Synergy rat unit; one SU is the minimum total quantity per rat, which when injected subcutaneously in generally divided doses, twice daily for three days into normal immature rats, produces in that time an average ovarian weight five times that of untreated controls) for two days to the intact or hypophysectomized animals. The control animals received only normal saline.

Part IV: The study of ovariectomy and subsequent administration of estradiol dipropionate on the oxygen consumption, phosphorus concentration and radiophosphorus incorporation of uteri. Estradiol-dipropionate (Di-ovocylin, Ciba) was injected intramuscularly into ovariectomized rats. Each rat received 50 µg. of drug daily for two days. All control animals were injected in the same way with equal volume of vehicle (0.05 ml. of sesame oil U.S.P.).

In all experiments except Part I, each rat was injected intraperitoneally with 50 microcuries of radioactive phosphorus, P^{32}, as phosphate solution (Atomic Energy of Canada, Ltd.) on the last day of treatment. The weight of the animal was recorded at the time of isotope injection and the animal was fasted for 24 hours until sacrificed.

C. Procedures.

1. Tissue Respiration.

The experimental rat was killed by decapitation and the blood sample was collected by inverting the neck over a 20 ml. beaker which contained two drops of heparin solution (Heparin Sodium Injection 1,000 U.S.P.). Usually two or three rats were sacrificed at one time and at least one was the control. The blood samples were collected separately and centrifuged immediately at 2,000 rpm for approximately 30 minutes. A longitudinal incision was made over the abdomen to the diaphragm and the ovaries and uterus were removed. The tissues were placed immediately in clean petri-dishes lined with KRP (127) moistened filter paper and kept in a moist, cold chamber (2-5° C). The ovaries were cleaned of adherent fat and oviducts. Two ovaries, in certain cases four ovaries, were carefully placed on each fiber-glass mat which was kept in moist petri-dishes in the cool chamber until all samples were ready for weighing. Since the rat has a duplex uterus, that is uterus with two cervixes, and completely separated uterine horns, the uterus was first cleansed of adherent fat and small blood vessels and a longitudinal incision was made through each uterine horn with a small scissor, and then transversely cut into small pieces about half a centimeter long. These were then carefully spread on the fiber-glass mat. Usually one uterine horn gave enough sample for one fiber-glass mat.

The tissue samples were weighed on a Gram-atic balance and placed on the removable trays in the Huston-Martin flasks of about 12 ml. capacity. The flask contained a piece of filter

paper moistened with 0.2 ml. of 20% potassium hydroxide on the bottom, upon which a removable tray containing 1.0 ml. of KRP solution was placed. The flasks were warmed to approximately 37° C in a water bath before the tissue samples were placed in them. After attaching to the manometer, the flasks were placed in the constant temperature bath at 37.9° C and flushed with pure washed oxygen for two minutes, then all ground glass connections were thoroughly tightened and 20 minutes was allowed for thermal equilibrium. The oxygen consumption was measured by the direct method of Warburg using a pure gas phase of oxygen. Manometric readings were taken at 20 minute intervals throughout the period of four hours. Usually it required about 25 to 30 minutes from the time of killing until the flasks were placed in the water bath.

The QO_2 used in this work expresses ml. of oxygen consumed per gm. of wet weight of tissue per hour. The QO_2 was plotted against the time interval on a graph paper. The QO_2 for zero time, which is believed to be the closest estimation of the respiration rate _in situ_ was obtained by straight line extrapolation of the plotted curve.

2. Phosphorus Determination and Radioassay.

The method for phosphorus determination and radioassay in this study was essentially the same as described by previous workers in this laboratory (30,31).

(a) Quantitative Determination of Phosphorus.

One ml. of plasma from each blood sample was removed after centrifugation and extracted three times with one ml. of ice-

cooled 10% trichloroacetic acid and the combined supernatants were diluted to ten ml. with demineralized distilled water.

The tissues were removed from the fiber-glass mat after four hours of respiration study and homogenized in one ml. of ice-cooled 10% trichloroacetic acid. The tube was centrifuged and the clear supernatant removed. This was done two more times. The combined supernatants were diluted to ten ml. with demineralized distilled water.

One ml. aliquots of the diluted extracts obtained from plasma, ovaries and uteri were wet ashed separately with 0.5 ml. of a mixture of 3 parts concentrated H_2SO_4 and 2 parts 60% $HClO_4$ in Kjeldahl flasks of 10 ml. or 30 ml. capacity on an Electrothermal Kjeldahl Apparatus. A blank containing 3.0 ml. of 10% trichloroacetic acid in 10 ml. of water and a standard solution containing 10 μg. phosphorus per ml. (as KH_2PO_4) were also wet ashed along with the tissue samples as described above. The changing of the ashed solution from colorless to a light yellow color and then returning to colorless indicated the completeness of ashing. The cooled ashed solution was diluted with 7.5 ml. of demineralized distilled water and neutralized with concentrated ammonium hydroxide using two drops of bromothymol blue as an indicator. The end point was reached when one drop of the base turned the yellow solution to blue color.

To each of the neutralized solutions from the wet ashing procedure 1.5 ml. of an acid molybdate color reagent was added. The solutions were placed in a boiling water bath for thirty minutes. The absorbance of the cooled solution was

measured in a Beckman Model B Spectrophotometer at 760 mμ. Distilled water was used as the reference solution. The value of the blank was subtracted from each reading before the concentration of the phosphorus in each sample was calculated. The concentration of phosphorus in each tissue was determined by the following equation:

$$C = \frac{AC_s}{A_s} \times \frac{D}{W}$$

where C = concentration of phosphorus in the tissue sample (μg/mg).
A = corrected optical density of the sample solution.
C_s = concentration of phosphorus in the standard solution (μg).
A_s = corrected optical density of the standard solution.
D = dilution factor.
W = wet weight of the tissue (mg).

(b) Determination of Radioactivity.

The colored solution prepared for the phosphorus determination was directly used in the radioassay.

The radioactivity of the sample was determined in an M-6 liquid Geiger-Müller counting tube (20th Century Electronics) attached to a decade scaler (Trace lab, Inc., Boston, Mass.). All counts were corrected for background, differences in the standard count, and for radioactive decay.

(c) Counting Error.

The standard error of a counting rate $n_s - n_b$ is:

$$\pm\sqrt{\left[\frac{\sqrt{N_s}}{t_s}\right]^2 + \left[\frac{\sqrt{N_b}}{t_b}\right]^2}$$

where N_s = total sample count.

t_s = total sample counting time.

N_b = total background count.

t_b = total background counting time.

$n_s = \frac{N_s}{t_s}$ = sample counting rate.

$n_b = \frac{N_b}{t_b}$ = background counting rate.

Each of the samples was counted for 3 minutes, and the background was counted for 10 minutes.

The uncorrected total sample count was never lower than 449, and the total background count was never more than 230. From these values, the standard error of the counting rate was found to be 126.7 ± 7.2 counts per minute. The percentage standard error was 5.68%. Since the net counting rate of most of the samples was greater than 126.7 counts per minutes, hence the percentage standard error was less than 5.68%.

(d) P^{32} Standard

The P^{32} standard was prepared by diluting the same amount of P^{32} solution used for the injection to 50 ml. with N/10 HCl in a volumetric flask. Triplicate 20 lambda aliquots were dried on planchettes and counted with an end window Geiger-Müller counting tube (Tracer lab.). The average counting rate

of the first dose injected in a series was assigned a value of 100%. Subsequent injections in the series were corrected to this standard dose. A simulated P^{32} standard (Tracer lab.) was also counted to correct for mechanical variations with the counting equipment.

D. Definition of Terms.

The specific activity (S.A.) of a sample is defined as the number of counts per minute per microgram of phosphorus.

$$\text{S.A.} = \frac{\text{Counts per minute}}{\text{Micrograms of phosphorus in the sample}}$$

Since each animal received 50 μc of P^{32} regardless of the weight of the animal, it is necessary to correct the specific activity of the plasma for differences in dilution. The specific activity of the plasma from a particular animal was multiplied by the body weight to give the corrected specific activity. For convenience, this value was multiplied by 10^{-2}.

$$\text{corrected S.A.} = \text{S.A. of plasma} \times \text{body weight} \times 10^{-2}$$

The relative specific activity (RSA) of a sample is defined as the S.A. of a sample relative to the corrected S.A. For convenience this value was multiplied by 10^{2}.

$$\text{R.S.A.} = \frac{\text{S.A. of the sample}}{\text{corrected S.A.}} \times 10^{2}$$

E. Statistical Analysis.

The mean QO_2, phosphorus concentration, specific activity and relative specific activity of each tissue from each series of experiments was taken for statistical analysis. Any differ-

ence from control values noted was tested for significance by the method of analysis of variance (128).

The probability of 0.05 was selected as the point of significance.

IV. RESULTS:

A. The effect of estrus and anestrus on the tissue respiration of sex organs in the female rat.

It was found that the oxygen consumption of ovaries obtained from animals during estrus was essentially the same as that found during anestrus. The mean QO_2 was 0.94 ± 0.13 for the estrus and 0.99 ± 0.11 for the anestrus group (Table I). The significance of the differences between means was tested using the analysis of variance. An F value of 1.57 was obtained. This value was far smaller than the F value required for significance at the 0.05 level (F 0.05 = 4.18), hence it was concluded that there is no significant difference between the oxygen uptake of ovaries obtained during estrus and anestrus.

The mean QO_2 of the uteri was 0.81 ± 0.09 for the estrus and 0.75 ± 0.11 for the anestrus group (Table I). In view of a higher uterine QO_2 obtained from the estrus group, the result led us to suspect that the oxygen uptake of the uterus might be somewhat enhanced under estrus conditions. Although a rather high F value was obtained (F = 3.98) yet this value was still insufficient to establish that the difference between the two means was significant at the 0.05 level.

B. The effect of norethindrone on the tissue respiration and phosphorus incorporation in the ovary and the uterus of the rat.

(1) Normal animals:

The oxygen consumption of the ovary was enhanced by norethindrone treatment (Fig. I). The mean QO_2 of the treated group was 1.12 ± 0.08 while that of the control group was 0.93 ± 0.01 (Table II). Comparison of the two means by analysis of variance gave an

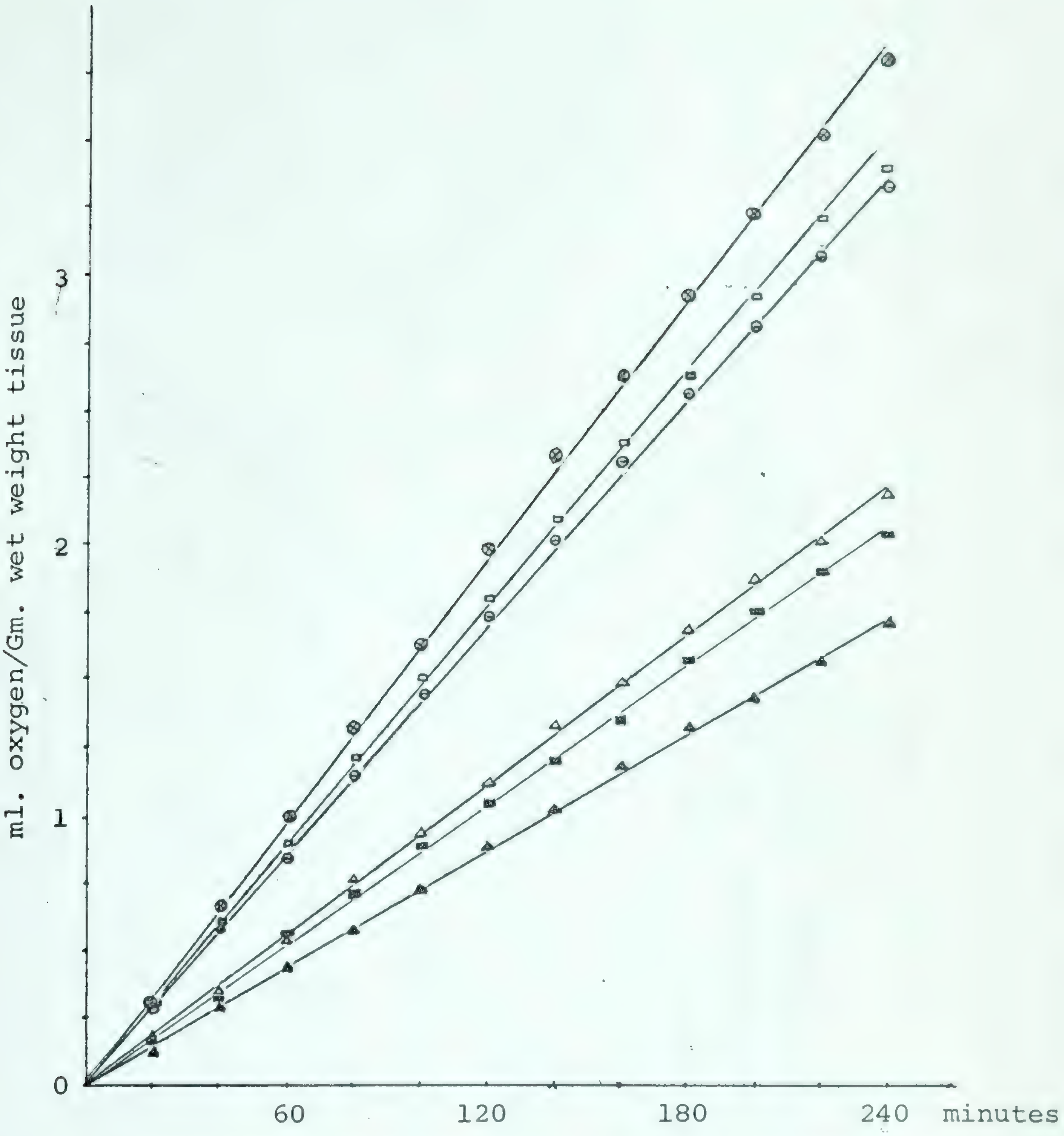


Fig. I. The effects of gonadotropin and norethindrone on oxygen uptake of the ovary of normal and hypophysectomized rats. Results for each group based on 7 to 17 experiments.

⊖——⊖ Normal
□——□ Normal + Gonadotropin
⊗——⊗ Normal + Norethindrone
△——△ Hypophysectomized
▲——▲ Hypophysectomized + Gonadotropin
■——■ Hypophysectomized + Norethyndrone

Table I. The Oxygen Consumption of Ovary and Uterus During Estrus and Anestrus in the Rat

Animal	Weight of Tissue ± S.D.*			Mean QO_2 ± S.D.			
Groups	Body (Gm)	Ovary (mg)	Uterus (mg)	Ovary	P**	Uterus	P
Estrus	198 ± 21 (11)***	57.4 ± 10.7 (10)	312.5 ± 60.5 (10)	0.94 ± 0.13 (10)	>0.05	0.81 ± 0.09 (18)	>0.05
Anestrus	182 ± 17 (23)	41.7 ± 6.7 (22)	252.9 ± 66.3 (20)	0.99 ± 0.11 (21)		0.75 ± 0.11 (31)	

* S.D. = Standard deviation of the mean.

** Significance is indicated between the groups by applying an analysis of variance.

*** Figures in parentheses indicate number of determinations.

Table II. The Effect of Norethindrone on the Oxygen Consumption of Ovary and Uterus of Normal and Hypophysectomized Rats

Animal Groups	Weight of Tissue $\pm$ S.D.			Mean QO_2 $\pm$ S.D.			
	Body (Gm)	Ovary (mg)	Uterus (mg)	Ovary	P	Uterus	P
Normal	247 $\pm$ 18 (4)	59.0 $\pm$ 13.0 (4)	320.7 $\pm$ 59.0 (4)	0.93 $\pm$ 0.01 (4)		0.95 $\pm$ 0.17 (4)	
Normal + Norethindrone	228 $\pm$ 16 (20)	51.8 $\pm$ 11.7 (20)	336.2 $\pm$ 18.7 (20)	1.12 $\pm$ 0.08 (7)	<0.01 <0.01	0.96 $\pm$ 0.14 (21)	<0.01
Hypophysectomized	163 $\pm$ 6 (9)	34.5 $\pm$ 4.1 (8)	107.1 $\pm$ 41.1 (8)	0.66 $\pm$ 0.05 (8)		0.49 $\pm$ 0.06 (9)	
Hypophysectomized + Norethindrone	164 $\pm$ 8 (14)	40.4 $\pm$ 5.4 (14)	167.5 $\pm$ 9.4 (14)	0.66 $\pm$ 0.05 (9)		0.67 $\pm$ 0.09 (14)	<0.01

F value of 21.59. The F value required for significance at 0.05 and 0.01 levels was 5.12 and 10.56, respectively. Hence the effect of norethindrone treatment on the QO_2 of the ovary can be considered as highly significant. The oxygen consumption of the uterus of the treated group was essentially the same as those of the control group (Fig. II). The mean QO_2 of the two groups was 0.95 ± 0.17 and 0.96 ± 0.14, respectively (Table II).

No significant changes were observed in the phosphorus concentration of the plasma, ovary and uterus (Table III, IV and V); and both the specific activity and the corrected specific activity remained fairly constant after treatment with norethindrone. The specific activity of the ovary from the treated group was found to be not significantly different from that of the control group. However, the relative specific activity of the two groups was significantly different at the 0.01 level, the treated group being higher (Fig. IV). Both specific activity and relative specific activity of the uterus were found to be significantly ($p < 0.01$, $p<0.05$) lowered after administration of norethindrone (Table V, Fig. IV).

(2) Hypophysectomized animals:

No alteration in the ovarian QO_2 was observed after administration of norethindrone to the hypophysectomized animal (Fig. I). The mean QO_2 of the treated group was 0.66 ± 0.05 and that of the control group was also 0.66 ± 0.05 (Table II). The oxygen consumption of the uterus was markedly enhanced by norethindrone treatment (Fig. II). The mean QO_2 of the treated group was 0.67 ± 0.09 and that of the control group was 0.49 ± 0.06 (Table II).

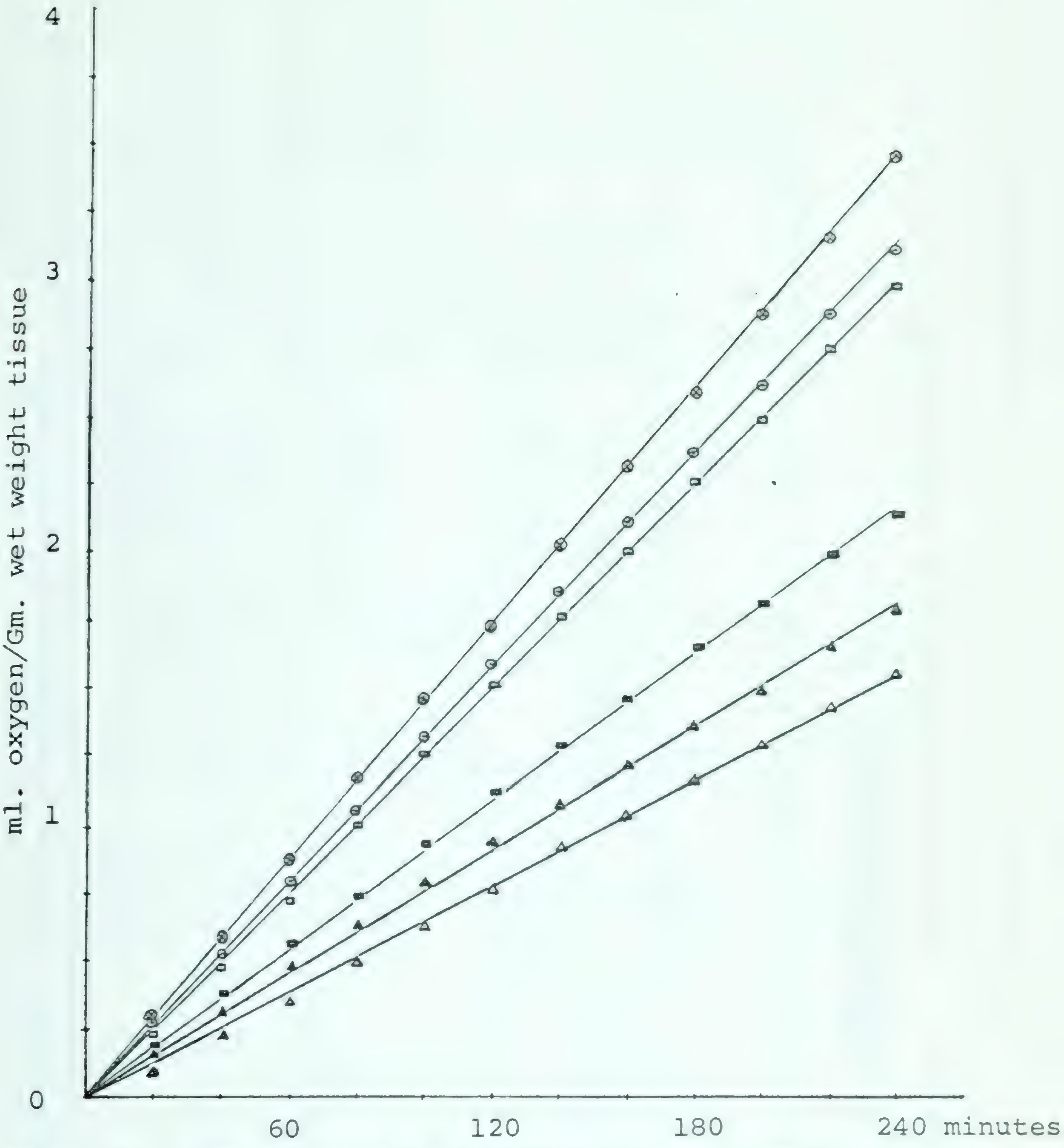


Fig. II. The effects of gonadotropin and norethindrone on the oxygen uptake of the uterus of normal and hypophysectomized rats. Results for each group based on 9 to 19 experiments.

⊖——⊖ Normal
▭——▭ Normal + Gonadotropin
⊗——⊗ Normal + Norethindrone
△——△ Hypophysectomized
▲——▲ Hypophysectomized + Gonadotropin
▬——▬ Hypophysectomized + Norethindrone

Table III. The Effect of Norethindrone on the Phosphorus Incorporation in the Plasma of Normal and Hypophysectomized Rats

Animal Groups	Phosphorus Concentrations ± S.D. (μg/ml)	P	Specific Activity* ± S.D.	P	Corrected Specific Activity** ± S.D.	P
Normal	59.8 ± 6.5 (8)	>0.05	40.2 ± 6.3 (8)	>0.05	98.7 ± 11.8 (8)	>0.05
Normal + Norethindrone	63.7 ± 6.0 (23)		35.8 ± 8.3 (23)		84.4 ± 20.9 (23)	
Hypophysectomized	64.8 ± 6.7 (16)	>0.05	49.2 ± 8.7 (16)	>0.05	80.3 ± 11.7 (16)	>0.05
Hypophysectomized + Norethindrone	70.0 ± 7.9 (26)		50.1 ± 8.0 (26)		81.4 ± 11.0 (26)	

* Specific Activity = $\frac{\text{counts/minute}}{\mu\text{g phosphorus in tissue}}$

** Corrected Specific Activity = Body weight x Specific Activity x 10^{-2}

Table IV. The Effect of Norethindrone on the Phosphorus Incorporation in the Ovary of Normal and Hypophysectomized Rats

Animal Groups	Phosphorus Concentration ± S.D. (μg/Gm)	P	Specific Activity ± S.D.	P	Relative Specific Activity* ± S.D.	P
Normal	1,100 ± 100 (8)	> 0.05	40.6 ± 9.6 (8)	< 0.01	40.1 ± 8.0 (14)	< 0.05 < 0.01
Normal + Norethindrone	1,130 ± 120 (22)		39.9 ± 9.3 (23)	< 0.01	48.8 ± 7.2 (19)	< 0.01
Hypophysectomized	1,200 ± 130 (18)	> 0.05	52.8 ± 9.5 (16)		66.3 ± 6.2 (16)	
Hypophysectomized + Norethindrone	1,200 ± 320 (26)		50.0 ± 7.8 (26)		62.0 ± 9.8 (26)	

$$* \text{ Relative Specific Activity} = \frac{\text{Specific Activity}}{\text{Corrected Specific Activity}} \times 10^{2}$$

Table V. The Effect of Norethindrone on the Phosphorus Incorporation in the Uterus of Normal and Hypophysectomized Rats

Animal Groups	Phosphorus Concentration ± S.D. (μg/Gm)	P	Specific Activity ± S.D.	P	Relative Specific Activity ± S.D.	P
Normal	600 ± 120 (12)		42.1 ± 6.3 (11)		44.8 ± 7.7 (16)	
Normal + Norethindrone	640 ± 120 (31)		33.2 ± 9.2 (29)		39.3 ± 7.7 (30)	<0.05 <0.05
Hypophysectomized	670 ± 60 (18)	<0.01 <0.05	40.0 ± 6.9 (14)	<0.01 <0.01	56.1 ± 9.7 (16)	<0.01 <0.01
Hypophysectomized + Norethindrone	860 ± 140 (24)		60.8 ± 9.2 (24)		75.5 ± 9.3 (23)	

It is of interest to note that the changes in phosphorus incorporation was well correlated to the changes in oxygen consumption in this series of experiments. Both the specific activity and the relative specific activity of the ovary from the norethindrone treated hypophysectomized animal showed no statistical difference in comparison to that of the control group (Table IV). In contrast, the specific activity and relative specific activity of the uterus were significantly higher than that found in the control group (Table V).

The phosphorus concentration of the plasma and the ovary remained fairly constant while uterine phosphorus concentration was significantly increased after administration of norethindrone (Fig. III and V).

No significant changes were observed in the specific activity and the corrected specific activity of the plasma in the norethindrone treated group.

C. The effect of gonadotropin on the tissue respiration and phosphorus incorporation in the ovary and the uterus of the rat.

(1) Normal animals:

Administration of gonadotropin to the normal animal exerts little effect on the oxygen consumption of the ovary and the uterus (Fig. I). The mean QO_2 of the ovary and uterus of the treated group were 0.99 ± 0.08 and 0.81 ± 0.08, whereas those of the control group were 0.96 ± 0.10 and 0.82 ± 0.08, respectively (Table VI). Statistical analysis showed no significant difference at the 0.05 level.

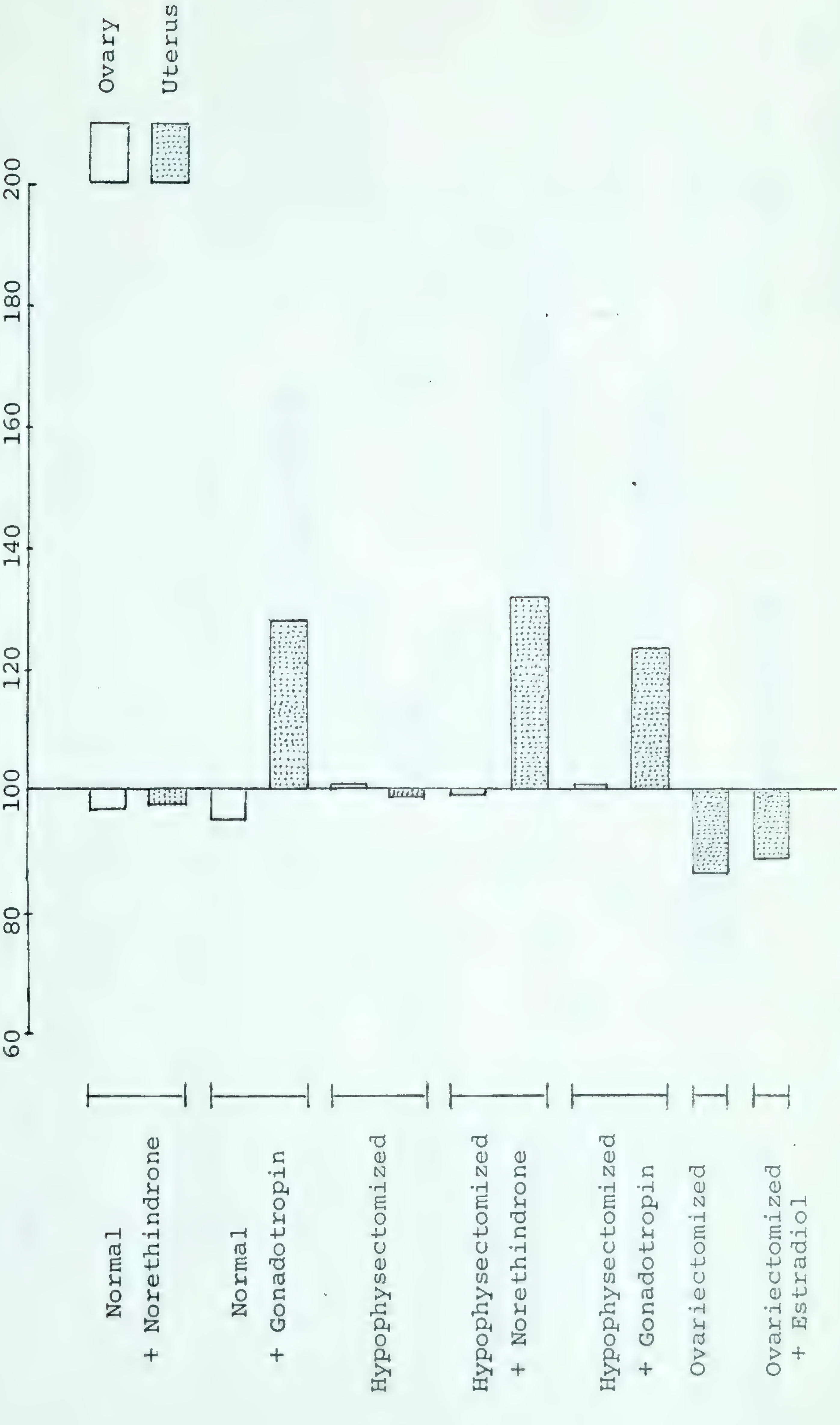


Fig. III. Phosphorus Concentration in Ovary and Uterus

Fig. IV. P^{32} Incorporation in Ovary and Uterus

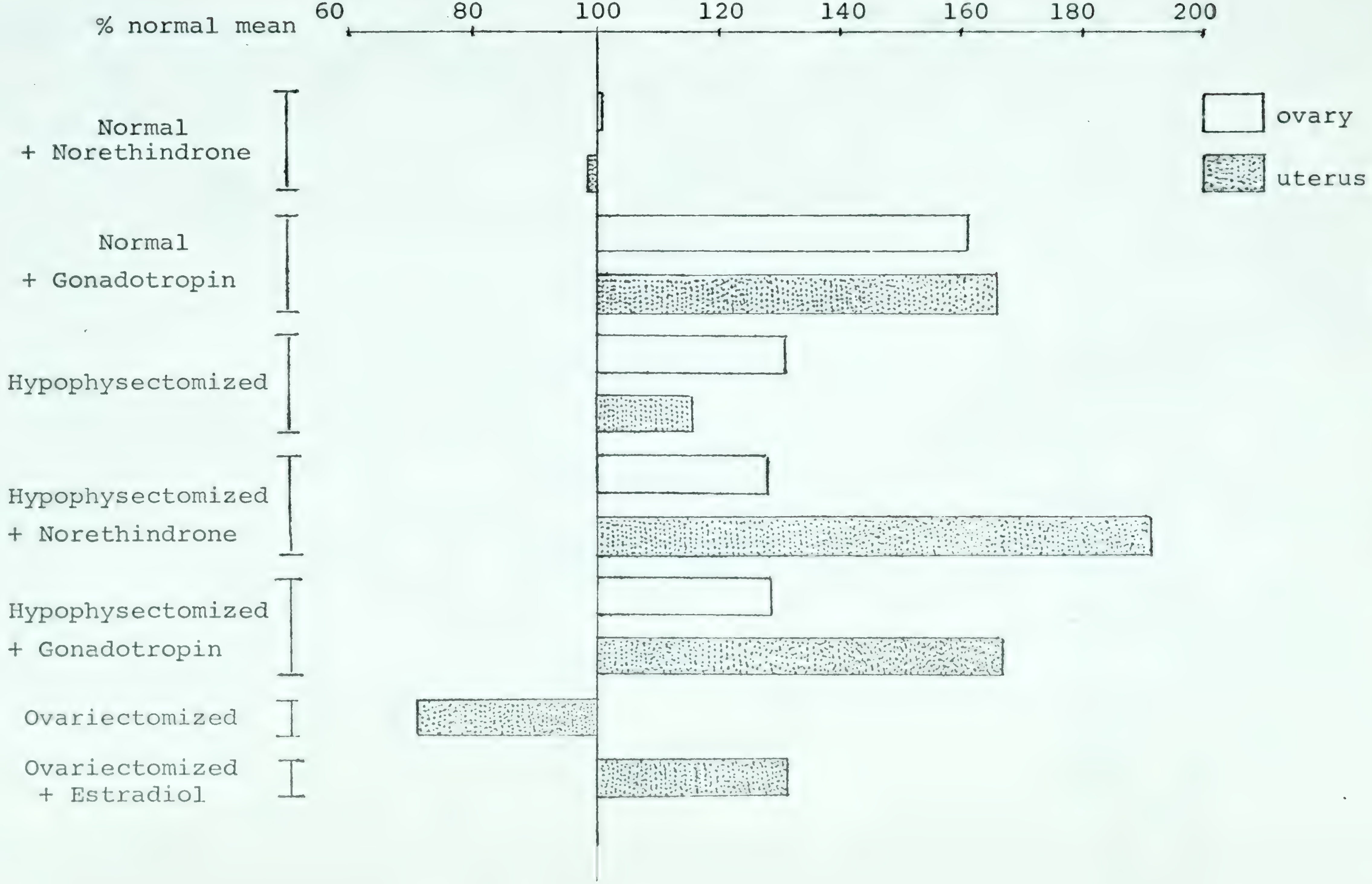


Fig. V.

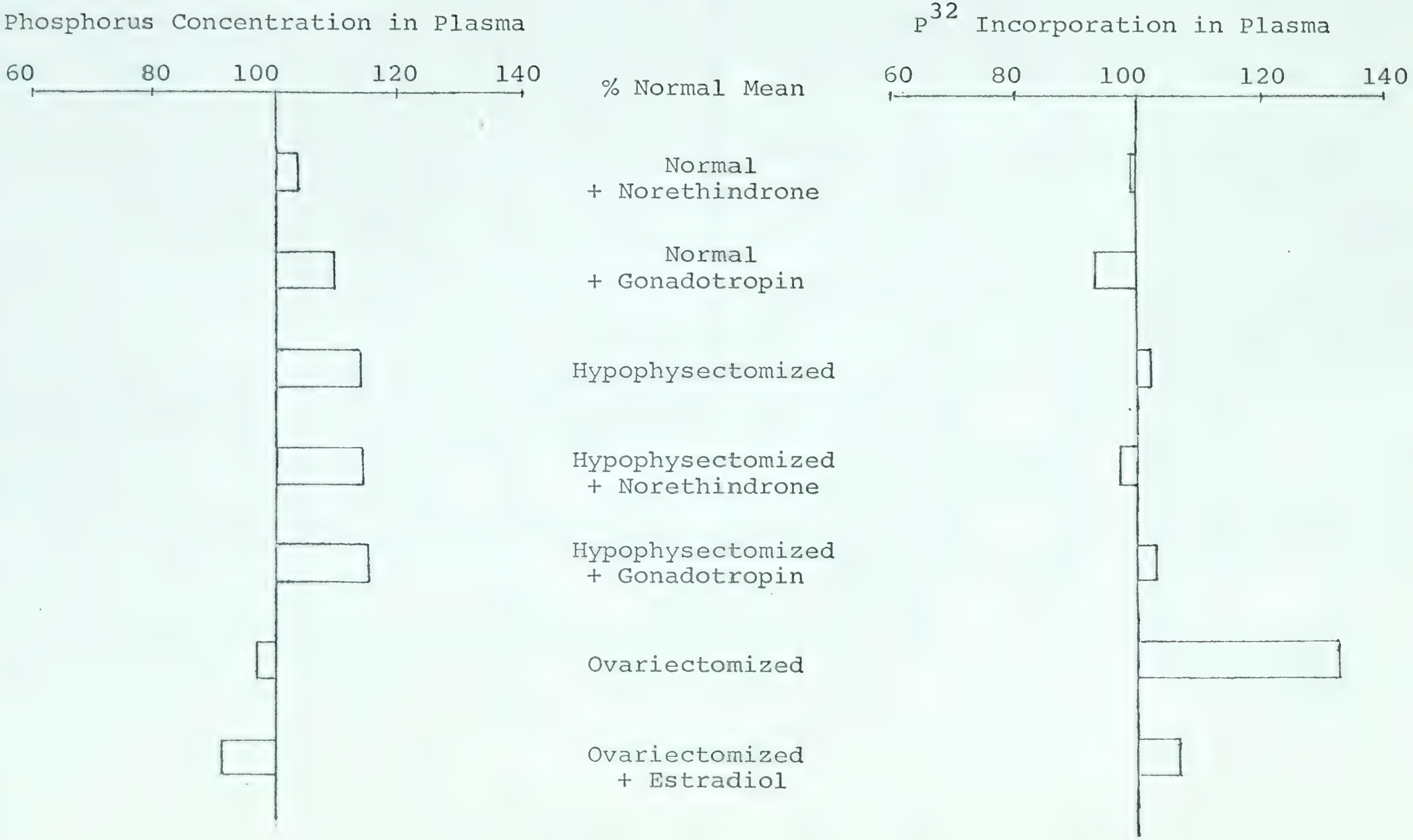

Phosphorus Concentration in Plasma
60
80
100
120
140
% Normal Mean
Normal + Norethindrone
Normal + Gonadotropin
Hypophysectomized
Hypophysectomized + Norethindrone
Hypophysectomized + Gonadotropin
Ovariectomized
Ovariectomized + Estradiol
P^{32} Incorporation in Plasma
60
80
100
120
140

Table VI. The Effect of Gonadotropin on the Oxygen Consumption of Ovary and Uterus of Normal and Hypophysectomized Rats

Animal	Weight of Tissue $\pm$ S.D.			Mean QO_2 $\pm$ S.D.			
Groups	Body (Gm)	Ovary (mg)	Uterus (mg)	Ovary	P	Uterus	P
Normal	209 $\pm$ 21 (9)	58.3 $\pm$ 18.2 (9)	306.8 $\pm$ 91.5 (9)	0.96 $\pm$ 0.10 (11)	<0.01	0.82 $\pm$ 0.08 (19)	>0.05
Normal + Gonado-tropin	207 $\pm$ 18 (17)	80.3 $\pm$ 20.6 (17)	344.8 $\pm$ 54.9 (17)	0.99 $\pm$ 0.08 (9)		0.81 $\pm$ 0.08 (13)	<0.01
Hypophys-ectomized	167 $\pm$ 13 (17)	44.9 $\pm$ 4.2 (14)	111.4 $\pm$ 17.9 (14)	0.67 $\pm$ 0.10 (17)	<0.01	0.46 $\pm$ 0.07 (18)	<0.01
Hypophys-ectomized + Gonadotro-pin	173 $\pm$ 14 (13)	69.5 $\pm$ 14.9 (13)	198.4 $\pm$ 31.1 (13)	0.56 $\pm$ 0.09 (12)	<0.05	0.54 $\pm$ 0.10 (14)	<0.05

The phosphorus concentration of the plasma and the ovary in the gonadotropin treated animal were found to be not statistically different from that of the control animal (Table VII, VIII). However, the phosphorus concentration of the uterus was significantly ($p < 0.01$) increased by gonadotropin treatment (Table IX and Fig. III).

Both the specific activity and the corrected specific activity of the plasma remained essentially the same after treatment with gonadotropin (Table VII).

Gonadotropin markedly enhanced the incorporation of the radiophosphorus into the acid soluble phosphate fraction of the ovary and the uterus. Both the specific activity and the relative specific activity of the ovary in gonadotropin treated groups were found to be significantly higher at the 0.01 level when compared to that of the control group (Table VIII). Although the specific activity of the uterus in the treated group was not significantly different at the 0.05 level, yet the relative specific activity was significantly different at the 0.01 level (Table IX).

(2) Hypophysectomized animals:

The QO_2 of the ovary of the hypophysectomized rat was found to be significantly higher than that of the gonadotropin treated rat (Table VI). However, the radiophosphorus uptake showed no change in the treated group (Fig. IV). Both the specific activity and the relative specific activity of the ovary remained essentially the same after treatment with 15 S.U. of gonadotropin (Table VIII and IX). It was somewhat surprizing that both the specific activity and the relative specific activity of the ovary

Table VII. The Effect of Gonadotropin on the Phosphorus Incorporation in the Plasma of Intact and Hypophysectomized Rats

Animal Groups	Phosphorus Concentration ± S.D. (μg/ml)	P	Specific Activity ± S.D.	P	Corrected Specific Activity ± S.D.	P
Normal	63.0 ± 7.0 (7)	>0.05	38.8 ± 7.9 (7)	>0.05	69.7 ± 9.6 (7)	>0.05
Normal + Gonadotropin	67.2 ± 5.8 (10)		38.9 ± 5.5 (10)		78.2 ± 11.7 (9)	
Hypophysectomized	75.7 ± 17.6 (18)	>0.05	54.2 ± 6.8 (18)	>0.05	92.5 ± 11.4 (18)	>0.05
Hypophysectomized + Gonadotropin	71.1 ± 12.2 (20)		53.8 ± 7.8 (20)		87.5 ± 13.2 (20)	

Table VIII.
The Effect of Gonadotropin on the Phosphorus Incorporation in the Ovary of Intact and Hyposphysectomized Rats

Animal Groups	Phosphorus Concentration ± S.D. (μg/ml)	P	Specific Activity ± S.D.	P	Relative Specific Activity ± S.D.	P
Normal	1,260 ± 290 (9)	>0.05	40.5 ± 9.4 (9)	<0.01	56.3 ± 5.8 (7)	<0.01
Normal + Gonado-tropin	1.110 ± 110 (10)		54.5 ± 9.1 (10)		77.9 ± 10.2 (8)	
Hypophys-ectomized	1,140 ± 70 (18)	>0.05	55.1 ± 8.0 (18)	>0.05	60.0 ± 8.6 (18)	>0.05
Hypophys-ectomized + Gonadotro-pin	1,190 ± 150 (20)		53.8 ± 7.8 (20)		61.7 ± 6.0 (20)	

Table IX. The Effect of Gonadotropin on the Phosphorus Incorporation in the Uterus of Intact and Hypophysectomized Rats

Animal Groups	Phosphorus Concentration ± S.D. (μg/Gm)	P	Specific Activity ± S.D.	P	Relative Specific Activity ± S.D.	P
Normal	700 ± 60 (7)	<0.05	35.1 ± 11.4 (7)	>0.05	34.5 ± 3.9 (6)	<0.01
Normal + Gonadotropin	830 ± 90 (10)		43.1 ± 4.4 (8)		66.1 ± 11.2 (8)	
Hypophysectomized	610 ± 30 (17)	<0.05	33.9 ± 7.1 (17)	<0.01	36.7 ± 8.4 (17)	<0.01
Hypophysectomized + Gonadotropin	800 ± 90 (20)		57.5 ± 9.4 (20)		65.8 ± 6.3	

obtained from the normal animal were significantly lower than that of the hypophysectomized or the gonadotropin treated hypophysectomized animal.

The phosphorus concentration in the plasma and the ovary remained essentially the same after gonadotropin treatment (Table VII and VIII). However, the phosphorus concentration in the plasma in this series was significantly higher than any other series studied. The corrected specific activity of the plasma from the gonadotropin treated hypophysectomized animal was significantly lower than that of the control series of animal (Table VII).

The QO_2 of the uterus of the hypophysectomized rat was found to be significantly lower than that of the gonadotropin treated rat (Table VI). These values were significantly lower than those of the intact animal (Fig. II).

The changes in radioactive phosphorus incorporation were correlated to the changes in the QO_2. Both the specific activity and the relative specific activity of the uterus from the gonadotropin treated hypophysectomized rat were significantly higher than that of the hypophysectomized control. In addition, the phosphorus concentration in the uterus was greatly enhanced by gonadotropin treatment (Table IX and Fig. III).

D. The effect of estradiol dipropionate on the oxygen consumption and phosphorus incorporation in the uterus of the ovariectomized rat.

The oxygen consumption of the uterus from the ovariectomized rat was significantly lower than the value obtained from the

normal rat, either during estrus or anestrus. Administration of 50 μg. of estradiol dipropionate daily for two days to the ovariectomized rat failed to bring back the normal rate of oxygen uptake in the uterus, but the QO_2 was significantly higher than that of the untreated animal (Table X and Fig. VI).

The changes in the radiophosphorus incorporation into the acid soluble fraction of the ovariectomized rat were very profound following estradiol dipropionate treatment. Both the specific activity and the relative specific activity showed differences which were highly significant when compared to the same measurement made on the untreated ovariectomized rat (Table XII). In addition, these values were higher than those obtained from the normal animal (Fig. III).

The phosphorus concentration in the plasma and uterus remained essentially the same after treatment with estradiol dipropionate (Table XI and XII). These values were somewhat lower when compared to those of the normal animal (Fig. III and V).

The specific activity of the plasma showed no significant change. In contrast, the corrected specific activity of the plasma obtained from the ovariectomized rat was significantly higher than that of the estradiol dipropionate treated group (Table XI). The corrected specific activity of the plasma obtained from the normal animal was significantly lower than either ovariectomized or estrogen treated ovariectomized animals (Fig. V).

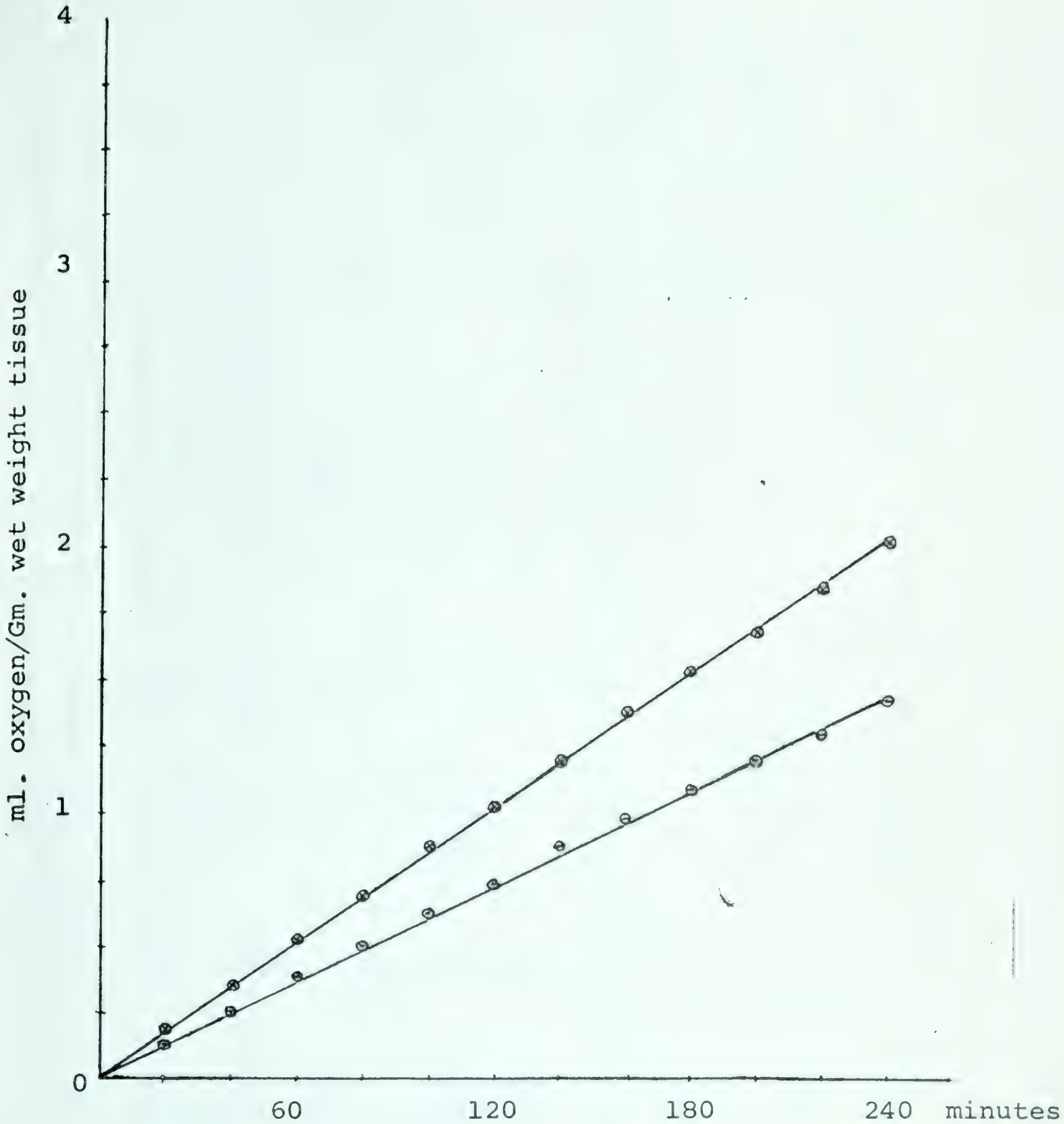


Fig. VI. The effect of estradiol-dipropionate on the oxygen uptake of uterus of ovariectomized rats. Results for each group based on 20 experiments.

○——○ Ovariectomized

⊗——⊗ Ovariectomized +
Estradiol-dipropionate

Table X. The Effect of Ovariectomy and Administration of Estradiol dipropionate on the Oxygen Consumption in Uterus of the Rat

Animal Groups	Weight of Tissue $\pm$ S.D.		Mean QO_2 of Uterus $\pm$ S.D.	P
	Body (Gm)	Uterus (mg)		
Ovariectomized	241 $\pm$ 31 (20)	108.6 $\pm$ 27.7 (17)	0.40 $\pm$ 0.11 (20)	<0.01
Ovariectomized + Estradiol dipropionate	231 $\pm$ 34 (25)	251.8 $\pm$ 40.0 (18)	0.61 $\pm$ 0.02 (36)	

Table XI. The Effect of Estradiol dipropionate on the Phosphorus Incorporation in the Plasma of Ovariectomized Rats

Animal Groups	Phosphorus Concentration ± S.D. (μg/ml)	P	Specific Activity ± S.D.	P	Corrected Specific Activity ± S.D.	P
Ovariectomized	59.6 ± 4.3 (32)	>0.05	47.2 ± 7.6 (27)	>0.05	112.2 ± 15.8 (29)	< 0.05
Ovariectomized + Estradiol dipropionate	55.9 ± 6.2 (36)		41.5 ± 6.7 (29)		90.3 ± 11.4 (23)	

Table XII. The Effect of Estradiol dipropionate on the Phosphorus Incorporation in the Uterus of Ovariectomized Rats

Animal Groups	Phosphorus Concentration ± S.D. (μg/Gm)	P	Specific Activity ± S.D.	P	Relative Specific Activity ± S.D.	P
Ovariectomized	560 ± 80 (32)	>0.05	33.2 ± 8.4 (25)	<0.05	28.6 ± 5.1 (25)	<0.01
Ovariectomized + Estradiol dipropionate	570 ± 50 (31)		44.6 ± 6.2 (24)		52.3 ± 8.8	

V. Discussion:

A. The Influence of estrus and anestrus on the respiration of sex organs.

It is well recognized that estrogen and progesterone play very important roles in the estrus cycle of an animal. Generally speaking, ovarian estrogen content is highest during estrus and lowest during anestrus, while the progesterone level varies in the opposite direction.

The results obtained in the present study clearly indicate that the metabolism of the ovary, as measured by this method of determining oxygen consumption, is fairly constant during estrus and anestrus. This suggests that despite the changes in the level of the endogenous ovarian hormones which are taking place in the ovary during estrus and anestrus, the ovary itself is little influenced. Since the ovary responds to the pituitary gonadotropin by secreting hormones which exert effects on the uterus, and to a lesser extent on the other accessory sex organs, these findings would suggest that the primary effect of the ovarian hormones is to be observed on target organs.

In our experiments, the oxygen consumption of the uterus obtained from the estrus rat was somewhat higher than that of the anestrus rat. Statistical analysis of the means of the two groups gave a result which came close to the 0.05 level of significance. Also, it was observed that there was a large variation in oxygen uptake within the anestrus group. The QO_2 ranged from 0.96 to 0.48. This large variation in metabolic rate of uterus from the anestrus animal has been previously reported by several workers (48,49,50). It is not surprising that the estrus group has a higher oxygen consumption. Stuermen and Stein (129)

have reported a very similar result in the study of oxygen consumption in human endometrium. They found that oxygen consumption rose during the late proliferative and late secretory phases. However, the differences were not pronounced and overlapping of values occurred. Statistical analysis showed no significant variation in the two groups. Our result also supports the finding of Hagerman and Villee (130), which demonstrated that a slight cyclic variation in average oxygen consumption was observed in human endometrium during the menstrual cycle.

The slight increase in the oxygen uptake of the uterus in estrus might be associated with the considerably higher level of estrogen secretion during this stage. As can be seen in the result obtained from the ovariectomized animals, exogenous estrogen definitely and markedly increased the oxygen consumption of the spayed rat uterus. Estrogens and certain steroid hormones have been shown to stimulate several enzyme systems *in vitro* (131). It may not be inappropriate to speculate that the changes in these enzyme systems as mediated by estrogens would alter the rate of some metabolic pathway to a certain extent, as reflected by the slight increase in utilization of oxygen.

B. The effects of norethindrone on the normal and hypophysectomized rat.

It is of interest to note that administration of norethindrone to the normal animal increased the oxygen consumption of the ovary and also the incorporation of P^{32} into the ovarian tissue. However, in the hypophysectomized animal, norethindrone had no effect on both parameters. The oxygen consumption and

inherent uterotropic action of norethindrone and its metabolite might contribute at least part of the stimulatory effect on the uterus to elevate the oxygen consumption and balance its depressive effect on the hypothalamo-hypophyseal ovarian axis. In the absence of the hypophysis, norethindrone can no longer exert its inhibitory effect on the uterus through the mechanism just mentioned, but its direct action on the uterus, that is the uterotropic effect, is still retained. Thus administration of norethindrone to the hypophysectomized female rat markedly enhanced the oxygen consumption and also the phosphorus incorporation in the uterus. From our finding, it seems that norethindrone exerts its effect on the uterus through two completely different mechanisms. One is indirectly via the hypothalamo-hypophyseal ovarian axis with resulting inhibition on the uterine activity. Another is exerted directly on the uterine tissue with resulting increased activity. In the presence of an intact hypophysis, the inhibitory action is superior to the stimulatory action, so that only the former can be observed. However, after removal of the pituitary, the inhibitory action is completely blocked, and one can observe the stimulatory action of the drug on the uterus.

C. The effects of gonadotropin on normal and hypophysectomized animals.

The stimulatory effect of gonadotropin on the female sex organs has been well established. Administration of gonadotropin to the immature or hypophysectomized animal promotes the growth and proliferation of the ovarian tissue. It could be expected that during such extensive proliferation, active synthesis of ovarian cell components would take place within the

tissue. The study of other pituitary trophic hormones such as ACTH, growth hormone, and TSH clearly reveals that these hormones increase the incorporation of amino acids into the tissues of specific target organs.

The result obtained from the present study showed that synapoidin, a preparation which combines pituitary and chorionic gonadotropin activity, markedly enhanced the incorporation of P^{32} into the acid soluble phosphate fraction of the ovary and uterus of the normal female rat, but it had little effect on the oxygen uptake of these tissues.

The stimulatory nature of gonadotropin might account for the increased uptake of phosphorus in the ovary. An extensive proliferation of the ovarian cells under the stimulation of gonadotropin would give rise to the increased secretion of the steroid hormones, estrogen and progesterone, which in turn would enhance the rate of metabolism in the uterus as revealed by the increased uptake of P^{32}.

The failure of synapoidin to increase the oxygen consumption of the ovary and uterus of the normal animal is not what would be expected. There are two possibilities that could account for this finding. First, the weight of the tissue might have an influence on the calculation of tissue respiration. In the synapoidin treated group, the wet weight of the ovary was significantly increased. Both ovary and uterus showed excessive hyperemia and were slightly edematous. It is not unlikely that the increase of the weight is brought about by the increase of tissue water content. Since the QO_2 in this study was based on the wet weight of the tissue, a low QO_2 value in the synapoidin treated group might be obtained.

The overall picture would be no alteration of the measurement of oxygen consumption in this group, even though the actual oxygen consumption might be enhanced by the stimulatory action of gonadotropin. The second possibility is that the measurement of oxygen consumption and the determination of phosphorus incorporation may measure a different biochemical metabolic rate in the ovary and the uterus. Ahrén and Kostyo have reported that in prepubertal rats administration of FSH and LH did not stimulate the oxygen consumption and glucose uptake of the ovary, but FSH did enhance the incorporation of alpha-amino-isobutyric acid into the ovarian tissue (94). Walaas has suggested that in uterine tissue, carbohydrate that is not converted into lactic acid could be accounted for approximately by oxygen consumption (68). If his assumption is correct, then the measurement of oxygen consumption by the present method would represent primarily the measurement of aerobic glycolysis under the condition in which the endogenous glycogen served as a substrate. The acid soluble phosphate fraction serves as a metabolic pool for the intermediate metabolites requiring phosphorus. This phosphorus fraction is obtained from carbohydrates, fatty acids, and proteins. The degree of incorporation of P^{32} into this fraction is the measurement of the turnover of phosphorus within this pool. The turnover of phosphorus is determined by various factors including the changes of certain enzyme systems which are responsible for the biochemical reactions involved, and the transfer which occurs between compartments. The knowledge of this highly integrated and complicated biochemical system is far from complete. Although this may

serve as a useful hypothesis, no definite conclusion can be made until more detailed information is available.

Hypophysectomy caused a sharp decrease in the oxygen consumption and the phosphorus incorporation in the ovary and uterus. It is obvious that after removal of the trophic hormone which is in charge of the regulation and maintenance of the target organs, the ovary and uterus will undergo atrophy and remain quiet as far as the active synthesis of various substances is concerned. This shortage of substrates and lack of metabolic activity gave rise to the decrease of oxygen consumption in the tissue. Administration of synapoidin into the hypophysectomized animal will restore this deficiency and bring back the normal picture in these tissues as revealed by the increase of oxygen consumption and the incorporation of P^{32} in the uterus. But the finding that the QO_2 of the ovary of synapoidin treated hypophysectomized animals was significantly lower than that found in the control is rather hard to explain. In addition, the phosphorus incorporation in the ovary remained fairly constant within both control and treated hypophysectomized animals, which makes the finding even more complicated. It seems that the oxygen consumption and the phosphorus incorporation are completely out of correlation. It has been reported that administration of chorionic gonadotropin to hypophysectomized rats resulted in a decrease of the number of ova which developed into primordial follicles and also caused a reduction of the rate of growth during the last stages of follicular development (131). Since the preparation used in our experiment contains a mixture of chorionic and pituitary gonadotropin, we might therefore

suspect that the low QO_2 and the lack of enhancement of phosphorus incorporation in ovarian tissue of the treated hypophysectomized animals were the contribution of the chorionic gonadotropin.

D. The effects of estradiol dipropionate on the ovariectomized rat.

The importance of the ovary in maintenance of the function of the uterus is well recognized. From the result of the present study, it is apparent that the uterine tissue is in its lowest metabolic state after removal of the ovary. Both oxygen consumption and phosphorus incorporation of the uterus were extremely low in comparison to those found in the normal animal. Administration of estradiol to the ovariectomized animal dramatically brought back the oxygen consumption to a higher level and the uptake of P^{32} was greatly enhanced and even slightly higher than that found in the normal animal. This clearly indicates that estrogen is necessary for the regulation of the uterine metabolic activity.

It is of interest to note that the oxygen consumption and phosphorus incorporation are well correlated to one another either in ovariectomized or estradiol treated ovariectomized animals. The results suggest that there may be a substance within the ovary which can alter the parallelism between oxygen consumption and phosphorus incorporation in uterine tissue. Once there is removal of this endogenous substance, that is after ovariectomy, the oxygen consumption and phosphorus incorporation correlate with one another. A similar situation is

obtained after estrogen administration. Progesterone has been shown to uncouple oxidation and phosphorylation in various mitochondrial preparations (132) and to accelerate the latent adenosine triphosphate activity in the liver mitochondria (133). It is quite likely that the presence of progesterone in the normal animal has contributed, at least in part, to the differences observed between oxygen consumption and phosphorus incorporation in uterine tissue. The changes of oxygen consumption and phosphorus incorporation might be associated with the function of the uterine muscle. It is well established that estrogen induces contraction in the uterus of ovariectomized rabbits (134). Furthermore, estrogen has been found to stimulate transhydrogenation (135) and induce a reduced pyridine nucleotide oxidase system in rat uterus (136). From the study of muscle contraction, it has been shown that the energy liberted on enzymatic cleavage of the energy-rich phosphate from ATP is directly utilized for muscle contraction. The results from the present study showed that estradiol greatly enhanced this source of energy as reflected by the increased incorporation of P^{32} into the acid soluble phosphate fraction of uterine tissue. In addition, the oxygen consumption was also increased by estradiol administration. This further indicates that estrogen may bring about increased contraction of the uterine muscle.

VI. SUMMARY AND CONCLUSIONS.

The aim of the present study was to investigate the effect of estrus and certain hormones on the tissue respiration and the phosphorus metabolism of the ovary and the uterus of female rats under various endocrine states. It was found that:

(1) The oxygen consumption of the ovary and the uterus were essentially the same in normal female rats during estrus and anestrus. A large variation in oxygen consumption was observed in the anestrus group.

(2) The ovarian respiration and the phosphorus metabolism of normal female rats were markedly enhanced by the administration of norethindrone as revealed by the increase in mean QO_2 and radioactive phosphorus incorporation. On the other hand, the uterine respiration showed little effect and the radioactive phosphorus incorporation into uterine tissue was depressed by such treatment.

(3) Gonadotropin had no effect on either the ovarian or uterine respiration in normal female rats, but it increased incorporation of radioactive phosphorus into these tissues.

(4) Hypophysectomy induced a lower rate of respiration and phosphorus metabolism in the ovary and the uterus.

(5) Norethindrone had no significant effect on the respiration and phosphorus metabolism of the ovary in hypophysectomized female rats. However, it enhanced the uterine oxygen consumption and increased the incorporation of radioactive phosphorus into the acid soluble phosphate fraction of the uterus.

(6) Adminstration of gonadotropin to the hypophysectomized female rat decreased the ovarian oxygen consumption but had no effect on the ovarian radioactive phosphorus uptake. Both the oxygen consumption and the radioactive phosphorus incorporation of uterine tissue from hypophysectomized female rats were greatly enhanced by gonadotropin.

(7) Ovariectomy caused a marked reduction in the rate of oxygen consumption and the degree of radioactive phosphorus incorporation into uterine tissue.

(8) Estradiol dipropionate accelerated the rate of tissue respiration and the radioactive phosphorus uptake in the uterus of ovariectomized rats.

(9) The significance of the above findings has been discussed.

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B29825